September 13, 2005

Via E-Mail
Dr. Barbara Shane
Executive Secretary for the NTP Board
NTP Liaison and Scientific Review
NIEHS
Research Triangle Park, NC 27709

Re: American Chemistry Council Draft Comments on NTP MIBK Report

Dear Dr. Shane:

The Ketones Panel[1] of the American Chemistry Council submits the following comments on NTP's draft report TR 538 on the Toxicology and Carcinogenesis Studies of Methyl Isobutyl Ketone (MIBK) in Rats and Mice. We believe the report for the most part, accurately reflects the findings of the studies; however, we believe that the narrative should be modified in some places for completeness and to improve the utility of the report for future users, such as researchers, regulators, risk managers, and the public. The areas for suggested improvement are presented in specific comments below.

Mouse Liver Tumors

The qualitative aspects of the observed liver tumor response should be described in more detail and further clarified to more fully reflect the findings of the study; for example, the hepatocellular tumor response is almost entirely the result of an increase in hepatic adenomas and not hepatic carcinomas. In addition, the text does not address the metastatic characteristics of the mouse liver tumors.

For male mice (p. 55), the information presented is accurate, but fails to note that there is no increase in carcinomas in male mice, and, in fact, there was an actual dose-related *decrease* in the incidence of carcinomas.

For female mice (p. 55), the text notes that female mice have a higher incidence of carcinomas than the control group, but this text fails to clarify that higher incidence was seen only at the highest dose, and it is not statistically significant. At this dose, the Maximally Tolerated Dose was exceeded, as indicated by the decreased [<10%] body weight gain staring as early as 30 weeks and throughout the remainder of the study (p. 51; p. 54, Fig. 4).

[1] The Panel is comprised of the following companies: The Dow Chemical Company, Eastman Chemical Company, ExxonMobil Chemical Company, and Shell Chemical LP

The text does not address the metastatic characteristics of the mouse liver tumors. For example, the number of hepatocellular carcinomas that metastasized to the lung in both male and female mice was lower in the MIBK treated groups than the controls (males = 14% control, 10% low-dose, 6% mid-dose and 10% high-dose; females = 8% control, 2% low-dose, 2% mid-dose and 2% high-dose).

Therefore, the hepatocellular tumor response is almost entirely the result of an increase in adenomas and not carcinomas. These findings argue against evidence of any tumor progression.

The discussion should include a statement of possible modes of action.
Data from other studies reviewed in the Introduction (pp. 19, 20) indicate that MIBK and its metabolites are potent inducers of hepatic mixed function oxidase enzymes. The mode of induction of liver adenomas in mice may be to increase the clonal expansion of endogenous preneoplastic foci, similar to some other hepatic enzyme inducers such as Phenobarbital (Klaunig, 1993 and Isenburg et al., 2001). There is no evidence in the mouse study of other possible modes of actions, such as genotoxicity, hepatoxicity, oxidative stress, or peroxisomal proliferation.

Male Rat Kidney Tumors

A more detailed discussion of the male rat kidney tumors is warranted to indicate that these tumors should be expected based on the known short-term effects of MIBK related to renal accumulation of alpha-2 microglobulin. The report minimizes the available information while giving undue credence to a minority view of the relationship of alpha-2 microglobulin and rat renal tumors.

1. **All the evidence indicates that the increase in male rat kidney adenomas is by an alpha-2-microglobulin-mediated mechanism.**

Page 62 concludes that "Additional research is needed to characterize the binding of methyl isobutyl ketone to alpha-2-microglobulin and to clarify the role of alpha-2-microglobulin in the observed tumor outcome in male rats in the current two year study." Although NTP did not conduct a specific assay for the presence of alpha-2-microglobulin, all the evidence reported by NTP from previous studies, from this study, indicates that alpha-2 microglobulin is the mode of action for renal tumor formation related to MIBK exposure.

The main findings in the draft report related to kidneys of male rats after 2 years of exposure to methyl isobutyl ketone (MIBK) were statistical increases in: mineralization of the papilla (referred to here as LPM – linear papillary mineralization) in all treated groups; renal tubule hyperplasia (a preneoplastic precursor of renal tubule tumor) in all treated groups after combination of single and step sections; and adenomas/carcinomas in the high dose after combination of single and step sections. LPM is a feature of α2u-globulin nephropathy (Hard et al., 1993), and is a definite indication of that male rat syndrome. It reflects the chronic outcome of preceding cell damage related to hyaline droplet formation, which commences shortly after the beginning of compound exposure, and would be expected to persist until the

levels of hepatic synthesis of α2u-g decline in late-mid to old age (Roy et al., 1983; Hard et al., 1993). Hyaline droplet formation in male F344 rats has been recorded in separate studies at earlier time-points after exposure to MIBK, firstly in a 14-week inhalation study at exposures of 250 and 1000 ppm (Phillips et al., 1987), and again in F1 males at 17-19 weeks of 2000 ppm exposure in a reproductive toxicity study (Nemec at al, 2004). The occurrence of hyaline droplet formation in subchronic studies, and LPM in the chronic studies, makes a strong case for α2u-g nephropathy as a mode of action underlying the development of renal tubule hyperplasia and renal tubule tumors in the chronic study. However, in the Discussion of the NTP report (p. 62) there seems to be an element of diffidence in accepting this as the *modus operandi* for the tumors, despite a scientific consensus supporting it.

Page 6; line 10: This sentence correctly notes that renal tumors were noted in "…all groups of exposed male rats." in the extended evaluation. This sentence gives the reader the impression that tumors were found only in the exposed groups based on the extended evaluation when in fact renal tumors were also noted in the control animals based on the extended evaluation. (p. 44, Table 5). Indeed, the incidence of adenomas in the mid exposure group (900 ppm) was less than the incidence in the control.

Page 44, Table 5: Tables should include the complete summation of morphology related tumors. For example, Table 5 should have a data line (granted it would be 0 for all groups) to indicate that no additional renal tubular carcinomas were noted in the evaluation of step sections. Based on the presentation in the current Table 5, the reader must deduce that extensive evaluation of renal tissue failed to identify a single additional renal tubule carcinoma. This point is made in a brief sentence found pages after the Table.

2. Melnick's speculation is given too much credence and related statements in the text should be revised.

Page 61, paragraph 2: The presentation of Melnick's speculation in the discussion, that "α2u-g may serve as a vector to increase the delivery of a toxicant or protoxicant to proximal tubular cells, so that nephrotoxicity occurs not from the abnormal accumulation and degradation of α2u-g, but because chemical levels are elevated in the renal tubules" is inappropriate (Melnick, 1992). This is a minority scientific viewpoint and the statement should be deleted from the Discussion or at least denote that is a minority view. There is now longstanding evidence to support the concept that lysosomal overload is the injurious factor for the proximal tubules. This, and the entire mode of action, has been given lengthy consideration by a Monographs Committee of IARC and accepted as the working hypothesis (IARC, 1999: Swenberg and Lehman McKeeman, 1999). In a critique of Melnick's hypothesis by Borghoff et al. (1993), the weight of evidence rebutting this proposal was addressed, including a preliminary study showing that *in vitro* exposure of male rat kidney cells to high concentrations of a chemical that binds to α2u-g, namely 2,4,4-trimethyl-2-pentanol, did not result in any evidence of cytotoxicity. However, α2u-g itself was shown to be toxic in a primary renal cell culture (Lipsky et al., 1986), and exposure of hamster embryo cells to the protein also produced a degree of toxicity (Oshiro et al., 1998). In addition, crystallization of the protein has been observed ultrastructurally in the phagolysosomes of

male rat proximal tubule cells in α2u-g nephropathy (Hard et al., 1993), and this would not be expected if the role of α2u-g was simply to concentrate the chemical in the target cell.

3. **Exacerbation of CPN in rats should be regarded as an adverse effect, but not an indicator per se of MIBK- induced toxicity.**

The incidence and severity of CPN was increased in the high-dose males, while the incidences were significantly increased in all exposed groups of females, but the severity only slightly increased (page 43, paragraph 2). Chemical exacerbation of CPN usually occurs in conjunction with the induction of α2u-g nephropathy (Hard et al., 1993), so this is not a surprising observation in the males. However, exacerbation of CPN by chemicals is not confined to those causing toxicity through the α2u-g mechanism (Eustis et al., 1994; Hard and Khan, 2004; Lock and Hard, 2004), or to male rats (Hard, 2002; Lock and Hard, 2004). Thus, the apparent increased incidence of CPN in the MIBK-treated female rats would be quite independent of the α2u-g process occurring in the male rat kidney (see page 62, paragraph 2).

Regardless of the apparent increases, exacerbation of CPN in rats should be regarded as an adverse effect, but not an indicator *per se* of chemically induced toxicity. This is because the incidence and severity of this spontaneous disease can be influenced by physiological factors (Hard and Khan, 2004). CPN can be modified by varying the protein content of the diet or the source of protein (Iwasaki et al., 1988; Masoro and Yu, 1989; Rao et al., 1993), by varying caloric intake (Keenan et al., 2000), and by male sex steroid manipulation (Baylis, 1994). Furthermore, CPN is characterized by a spectrum of histopathology and clinical features that set it apart from the main causes of chronic renal disease in humans (Hard and Khan, 2004). Thus, rat CPN has no strict counterpart in humans and therefore appears to have no relevance to human hazard assessment (Hard and Khan, 2004).

4. **Transitional epithelial hyperplasia is a reflection of enhanced CPN, and should not be considered as an independent indication of kidney toxicity.**

Increased incidences of transitional epithelial hyperplasia in the renal pelvis were recorded in male rats exposed to 900 and 1800 ppm MIBK (page 46, paragraph 2). Although this same paragraph acknowledges that increased transitional cell hyperplasia can reflect enhanced CPN, this finding is restated as though possibly unrelated to CPN in the Discussion on page 62, paragraph 1. Transitional cell hyperplasia of the lining of the renal pelvis is a known accompaniment of advanced cases of CPN (Montgomery and Seely, 1990; Hard and Khan, 2004) and should be regarded as part of the histopathological spectrum of CPN.

5. **Many other structurally-related chemicals cause male rat kidney effects by an alpha-2-microglobulin mechanism.**

Many branched alkane hydrocarbons, and their oxygenated metabolites structurally similar to MIBK, cause hyaline droplet nephropathy in male rats, and may also have been shown to cause kidney tumors and/or alpha-2-microglobulin (see table). It is entirely expected that

MIBK acts by such a mechanism. The stated necessity to demonstrate binding of MIBK [or its metabolites] to alpha-2-microglobulin, as suggested (p. 62), is questioned.

Some Oxygenated Chemicals Which Cause Male Rat Kidney Adenomas

CHEMICAL	HYALINE DROPLETS	LINEAR MINERALIZATION	ALPHA-2-U GLOBULIN	MALE RAT KIDNEY ADENOMAS	CITATION
Methyl-isobutyl ketone [MIBK, 4-methyl-2-pentanone]	Y	Y	?	Y	This study
4-hydroxy-4-methyl-2-pentanone (HMP, metabolite of MIBK]	Y	?	?	?	MIBK draft SIAR
t-Butyl Alcohol	Y	Y	Y	Y	Lock and Hard, 2004
Methyl t-butyl ether [MTBE]	Y	Y	Y	Y	Bird et al., 1997
Propylene Glycol t-butyl Ether [PGBE]	Y	Y	Y	Y	Doi et al., 2004
Limonene oxide	Y	Y	Y	Y	Lock and Hard, 2004

*****

Summary

Consideration of the specific comments above, and suitable modification of the text, would allow future users of this report to more accurately assess the hazard to humans of exposures to MIBK.

Please contact Mr. William Gulledge, Manager of the Ketones Panel for further information. Bill can be reached at (703) 741-5613 or at william_gulledge@americanchemistry.com.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

References

Baylis C (1994). Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. J Clin Invest 94: 1823-1829.

Bird et al. (1997) Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats J Appl Toxicol. May;17 Suppl 1:S45-55

Borghoff SJ, Lehman-McKeeman LD, Short BG, Hard GC, and Swenberg JA (1993). Critique of R Melnick's "An Alternative hypothesis on the role of chemically induced protein droplet (α2u-globulin) nephropathy in renal carcinogenesis". Reg Toxicol Pharmacol 18: 357-364.

Doi et al., (2004) Inhalation toxicology and carcinogenesis studies of propylene glycol mono-t-butyl ether in rats and mice. Toxicology. 2004 Jun 1;199(1):1-22.

Eustis SL, Hailey JR, Boorman GA, and Haseman JK (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. Toxicologic Pathol 22: 457-472.

Hard GC (1990). Tumours of the kidney, renal pelvis and ureter in laboratory animals. In: Pathology of Tumours in Laboratory Animals, Vol 1 – Tumours of the Rat. V Turusov and U Mohr (eds), International Agency for Research on Cancer, Lyon. IARC Scientific Publications No 99, pp 301-344.

Hard GC (1999). Comparative kidney carcinogenesis in laboratory rodents. In: Carcinogenicity. Testing, Predicting, and Interpreting Chemical Effects. KT Kitchen (ed), Marcel Dekker Inc, New York, pp 439-466.

Hard GC (2002). Significance of the effects of ethyl benzene in rodents for assessing human carcinogenic risk. Toxicol Sci 69: 30-41.

Hard GC and Butler WH (1970). Cellular analysis of renal neoplasia: light microscope study of the development of interstitial lesions induced in the rat kidney by a single carcinogenic dose of dimethylnitrosamine. Cancer Res 30: 2806-2815.

Hard GC and Butler WH (1971). Ultrastructural study of the development of interstitial lesions leading to mesenchymal neoplasia induced in the rat renal cortex by dimethylnitrosamine. Cancer Res 31: 337-347.

Hard GC and Khan KN (2004). A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicologic Pathol 32: 171-180.

Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, and Valcovic LR (1993). Hazard evaluation of chemicals that cause accumulation of α_{2u}-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ Hlth Perspect 99: 313-349.

Hard GC, Alden CL, Stula EF, and Trump BF (1995). Proliferative lesions of the kidney in rats. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, DC, pp1-19.

IARC (1999). Consensus report. In: Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. CC Capen, E Dybing, JM Rice, and JD Wilbourn (eds), International Agency for Research on Cancer, Lyon. IARC Publications No 147, pp 95-118.

Isenberg JS, et al. 2001 Reversibility and persistence of di-2-ethylhexyl phthalate (DEHP)-and phenobarbital-induced hepatocellular changes in rodents. Toxicol Sci.; 64(2): 192-9.

Iwasaki K, Gleiser CA, Masoro EJ, McMahan CA, Seo EJ, and Yu BP (1998). The influence of dietary protein source on longevity and age-related disease processes of Fischer rats. J Gerontol43: B5-12.

Keenan KP, Coleman JB, McCoy CL, Hoe CM, Soper KA, and Laroque P (2000). Chronic nephropathy in ad libitum overfed Sprague-Dawley rats and its early attenuation by increasing degrees of dietary (caloric) restriction to control growth. Toxicologic Pathol 28: 788-798.

Klaunig, JE 1993 Selective induction of DNA synthesis in mouse preneoplastic and neoplastic hepatic lesions after exposure to phenobarbital. Environ Health Perspect. 101; l 5:235-9.

Lipsky M, Alden CL, and Gorton CP (1986). In vitro studies of hydrocarbon-induced male rat nephropathy. Toxicologist 6: 89 (abstract).

Lock EA and Hard GC (2004). Chemically induced renal tubule tumors in the laboratory rat and mouse: review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information. Crit Rev Toxicol 34: 211-299.

Masoro EJ and Yu BP (1989). Diet and nephropathy. Lab Invest 60: 165-167.

Melnick RL (1992). An alternative hypothesis on the role of chemically induced protein droplet (α_{2u}-globulin) nephropathy in renal carcinogenesis. Reg Toxicol Pharmacol 16: 111-125.

Nemec MD, Pitt JA, Topping DC, Gingell R, Pavkov KL, Rauckman EJ, and Harris SB (2004). Inhalation two-generation reproductive toxicity study of methl isobutyl ketone in rats. Int J Toxicol 23: 127-143.

NTP (1998). Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program, NIEHS, Research Triangle Park, NC. NTP Technical Report No. 467.

NTP (2005). Toxicology and Carcinogenesis Studies of Methyl Isobutyl Ketone (CAS No. 108-10-1) in F344 Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program, NIEHS, Research Triangle Park, NC. NTP Technical Report No. 538, Draft.

Oshiro Y, Balwierz PS, Eurell TE, Morris DL, and Alden CL (1998). Exploration of the transformation potential of a unique male rat protein alpha$_{2u}$-globulin using hamster embryonic cells. Toxicologic Pathol 26: 381-387.

Phillips RD, Moran EJ, Dodd DE, Fowler EH, Kary CD, and O'Donaghue J (1987). A 14-week inhalation toxicity study of methl isobutyl ketone. Fundam Appl Toxicol 9: 380-388.

Rao GN, Edmondson J, and Elwell MR (1993). Influence of dietary protein concentration on severity of nephropathy in Fischer-344 (F-344/N) rats. Toxicologic Pathol 21: 353-361.

Roy AK, Nath TS, Motwani NM, and Chatterjee B (1983). Age-dependent regulation of the polymorphic forms of α_{2u}-globulin. J Biol Chem 258: 10123-10127.

Swenberg JA and Lehman-McKeeman LD (1999). α_2-Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. In: Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. CC Capen, E Dybing, JM Rice, and JD Wilbourn (eds), International Agency for Research on Cancer, Lyon. IARC Publications No 147, pp 95-118.

Wilke AV, Dorman DC, and Borghoff SJ (1993). α2u-Globulin (α2u) and 2,4,4-trimethyl-2-pentanol (TMP-2-OH) toxicity in proximal tubule cells. Toxicologist 13: 439 (abstract).